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INVITED REVIEW

NADPH oxidases in cardiovascular disease: insights from in vivo models and clinical studies

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Abstract NADPH oxidase family enzymes (or NOXs) are the major sources of reactive oxygen species (ROS) that are implicated in the pathophysiology of many cardiovascular diseases. These enzymes appear to be especially important in the modulation of redox-sensitive signalling pathways that underlie key cellular functions such as growth, differentiation, migration and proliferation. Seven distinct members of the family have been identified of which four (namely NOX1, 2, 4 and 5) may have cardiovascular functions. In this article, we review our current understanding of the roles of NOX enzymes in several common cardiovascular disease states, with a focus on data from genetic studies and clinical data where available.

Keywords NADPH oxidase · Cardiovascular · Redox signaling · Cardiac hypertrophy · Hypertension · Atherosclerosis

Introduction

Reactive oxygen species (ROS) have long been known to be capable of inducing detrimental effects through the oxidation and damage of cellular macromolecules,

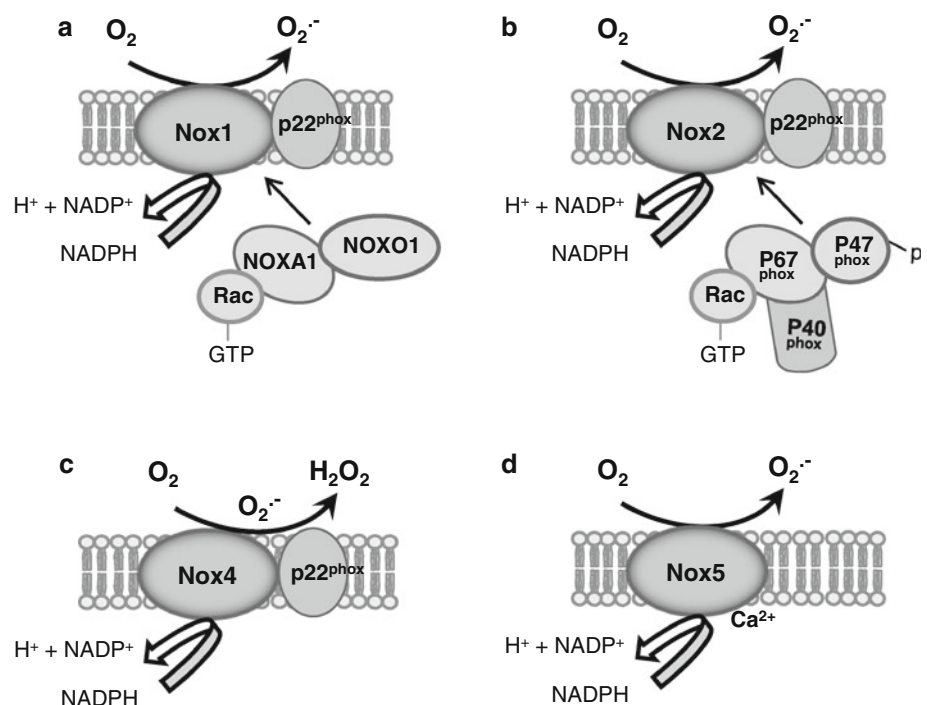
membranes, proteins and DNA, when they overwhelm endogenous antioxidant defences—a condition known as oxidative stress. More recently, however, it has been appreciated that they also modulate specific cellular signalling pathways through targeted effects on susceptible molecules, especially when generated in lower amounts—an action that is termed “redox signalling” [25, 56]. Redox signalling and oxidative stress are important in numerous cardiovascular conditions such as atherosclerosis, hypertension and heart failure [28, 30, 72, 85].

Potential sources of cellular ROS include mitochondria, NADPH oxidase family enzymes (or NOXs), dysfunctional nitric oxide (NO) synthases, xanthine oxidase and other oxygenases. Among these sources, the NOXs are unique in that ROS production is their primary function rather than a secondary effect [7]. Furthermore, they appear to be especially important in the modulation of redox-sensitive signalling pathways that underlie key cellular functions such as growth, differentiation, migration and proliferation. A large body of work published over the last decade indicates that NOX enzymes play important roles in the pathophysiology of many cardiovascular diseases [12, 16, 69]. Over this period, much has been learnt about the complexity of these enzymes, their regulation and their involvement in specific pathological processes. The most compelling data derive from studies that have used gene-modified animal models or specific genetic approaches to define the functions of these enzymes since chemical inhibitors of the enzymes lack specificity [2, 47]. Here, we provide a brief review of the current understanding of the roles of NOX enzymes in several common cardiovascular disease states, with a focus on data from genetic studies. We also review clinical data supporting a role for NOXs, where available.

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Fig. 1 Schematic representation of NOX1, NOX2, NOX4 and NOX5 oxidases. NOX1 and NOX2 require association with cytosolic regulatory subunits for their activation, as indicated



NADPH oxidase structure and regulation

The NOX family oxidases comprise seven members, each based on a distinct core catalytic subunit; i.e. NOX1–5 and DUOX1–2 [7, 66]. All NOX enzymes utilize NADPH as an electron donor and catalyse transfer of electrons to molecular oxygen to generate superoxide ($O_2^{\cdot-}$) and/or hydrogen peroxide (H_2O_2). NOX 1, 2, 4 and 5 may be expressed in cardiovascular cells although NOX5 is only found in humans and not rodents. The enzymes have differing requirements for other subunits and also exhibit differences in activity (Fig. 1; Table 1). NOX1, 2 and 4 all bind to a smaller p22^{phox} subunit, which is essential for enzyme activity. NOX1 and 2 are normally activated after cell stimulation by specific agonists (e.g. G-protein coupled receptor agonists such as angiotensin II, growth factors, cytokines and mechanical forces), which induce the association of regulatory subunits and activation of the enzyme. In the case of NOX2, the cytosolic regulatory subunits are p47^{phox}, p67^{phox}, p40^{phox} and Rac1 whereas NOX1 binds to the analogues of p47^{phox} and p67^{phox} termed NOXO1 and NOXA1, respectively, as well as Rac1. In marked contrast, NOX4 is constitutively active and does not require association with regulatory subunits, with regulation thought to occur mainly by changes in expression level. Furthermore, recent findings from several laboratories intriguingly suggest that NOX4 predominantly generates H_2O_2 in contrast to NOX1 and 2, which generate $O_2^{\cdot-}$ [21, 83, 103]. The biochemical basis for this property of NOX4 has recently been suggested to be related to the structure of its third extracytosolic loop, which differs

significantly from those of NOX1 or 2 and contains a highly conserved histidine moiety which may prevent superoxide release or may provide protons for superoxide dismutation [108]. NOX5 is regulated by calcium binding as a consequence of EF motifs in its N-terminal domain.

NOX1 is expressed mainly in vascular smooth muscle cells (VSMC) although endothelial cell expression has also been reported. NOX2 (also known as gp91^{phox} oxidase) was the first NADPH oxidase to be identified, being responsible for the phagocytic oxidative burst of neutrophils. Its neutrophil activity is important for non-specific host defence against microbial organisms and deficient activity of the oxidase results in chronic granulomatous disease (CGD), a condition in which affected children develop recurrent infections and inflammation. NOX2 has subsequently been found to be expressed at lower level in other inflammatory cells as well as in endothelial cells, cardiomyocytes, fibroblasts and VSMC. NOX4 is reported to be expressed in all cardiovascular cell types although its *in vivo* level of expression in healthy cardiovascular tissues remains to be precisely defined. NOX5 is reportedly expressed in human endothelial cells and VSMC although the data supporting its involvement in cardiovascular pathology are much weaker than that for the other NOX isoforms.

Hypertension

NOXs are expressed in all layers of the vessel wall as well as in other key organs, such as the kidney and the central

Table 1 The activity, regulation and expression of the main NOXs in the cardiovascular system

	NOX1	NOX2	NOX4	NOX5
Constitutive activity	Absent or very low	Absent or very low	High	Low
Requirement for p22 ^{phox}	Yes	Yes	Yes	No
Essential regulatory subunits	NOXO1, NOXA1, Rac	p67 ^{phox} , p47 ^{phox} , p40 ^{phox} , Rac	None	None
Control	Post-translational modifications of regulatory subunits	Post-translational modifications of regulatory subunits	Transcriptional. Can be regulated by Poldip2	Calcium binding
Cell expression	Vascular smooth muscle, possibly endothelial cells	Endothelial cells, cardiomyocytes, fibroblasts, human vascular smooth muscle, inflammatory cells	Endothelial cells, cardiomyocytes, fibroblasts, vascular smooth muscle cells	Human endothelium, human vascular smooth muscle

nervous system (CNS), that affect the regulation of blood pressure (BP) [7, 52, 68, 94].

At the level of the vessel wall, ROS may affect vascular resistance either by direct modulation of vessel tone or by affecting vascular remodelling [92]. Vascular O₂^{•-} production can disrupt physiological NO signalling through its inactivation and the production of peroxynitrite, resulting in a net vasoconstrictor effect. However, H₂O₂ has vasodilator actions through several mechanisms and could potentially reduce vessel tone. NOX-modulated alterations in activation of redox-sensitive signalling pathways that are involved in the vascular remodelling characteristic of chronic hypertension are also important [10]. Recently, evidence was reported that NOX2 activation in inflammatory cells (in particular, T-cells) that infiltrate the vessel wall is important in angiotensin II-dependent hypertension [43].

At renal level, ROS-modulated mechanisms have been shown to operate downstream of major determinants of blood pressure, namely hormonal regulation and sodium balance. Angiotensin II-mediated experimental hypertension is associated with oxidative stress [97] and ameliorated by treatment with superoxide dismutase [71]. Angiotensin II infusion is associated with an upregulation of NADPH oxidase activity in the renal cortex in rat and rabbit models [17, 78, 116, 117], with upregulation of NOX1 mRNA expression and downregulation of NOX4 mRNA expression [17]. In contrast, a high salt intake suppresses the renin–angiotensin–aldosterone axis and yet is also associated with an increase in NADPH oxidase activity in the renal cortex, accompanied by upregulation of gp91 (NOX2) and p47^{phox} mRNA [63]. A low salt intake produces the opposite changes. Hence there is a distinct pattern of activation of NADPH oxidase isoforms in the kidney in response to these different stimuli which are of known importance in the long-term control of blood pressure.

Finally, at CNS level, ROS are involved in modulating neuronal outputs that affect the central control of BP. Angiotensin II in the circulation is detected by certain areas of the brain outside the blood–brain barrier, including the subfornical organ (SFO). Studies by the group of Davisson showed that ROS appear to be responsible for mediating key central actions of Angiotensin II, including effects on blood pressure and thirst [125, 126]. Using adenoviral-based inhibition or overexpression of Rac1, this group also demonstrated that a Rac1-dependent NADPH oxidase (presumably NOX2) is the critical source for this ROS [124].

As suggested by the data presented above, the influence of NOXs on blood pressure has been most extensively studied in the models of angiotensin II-induced hypertension. In rodents, angiotensin II increases NOX-dependent ROS production in vessels, kidneys and CNS [17, 19, 33, 67, 77, 97, 115, 125]. This is associated with increases in NOX subunit expression and activity in all cell types within the vessel wall. Studies using systemic infusion of the peptide gp91-dstat (which inhibits activation of NOX2 and possibly other NOX isoforms) showed a significant attenuation of angiotensin II-induced hypertension and superoxide production in mice [99]. Use of siRNA targeted against p22^{phox} in rats was reported to reduce renal cortex NOX1, 2 and 4 expression and overall renal NADPH oxidase activity and to significantly reduce the pressor response to angiotensin II [87]. Additional data derive from the studies in gene-modified mouse models. Mice with a VSMC-specific overexpression of p22^{phox} did not develop hypertension, although interpretation of the results was potentially confounded by concurrent major changes in the expression of antioxidant genes and NO synthase [70]. Mice lacking the organizer subunit p47^{phox} showed markedly blunted pressor responses to angiotensin II [67]. This effect could involve NOX1 or NOX2, both of which may be affected by p47^{phox} deficiency. Indeed, mice with a

global knockout of NOX1 showed significant attenuation of pressor responses to angiotensin II infusion [22, 36, 84], whereas mice overexpressing NOX1 in VSMC showed exaggerated BP responses compared to wild-type mice [22, 84]. In contrast, mice with a global deficiency of NOX2 were reported to have an unaltered BP response to angiotensin II [116], suggesting that this isoform is less important. Similarly, crossing a global NOX2 knockout with mice that had chronic angiotensin II-dependent hypertension due to transgenic liver expression of human renin failed to affect BP [111]. While NOX4 levels are known to be increased in models of renin-angiotensin II-dependent hypertension [119], its possible role in the genesis of the hypertension has been unclear. Recent studies in a model of endothelial-specific NOX4 overexpression showed that NOX4 enhanced endothelium-dependent relaxation as a result of H_2O_2 -dependent vasodilatation [98], opposite to the effects of NOX1 or NOX2. This effect involved H_2O_2 -dependent hyperpolarization rather than a change in NO bioactivity. In addition to the enhanced vasodilatation, NOX4 transgenic mice had a lower BP than wild-type littermates and displayed a blunted hypertensive response to angiotensin II infusion [98]. These data suggest that elevation of NOX4 could act to counteract the hypertensive effects of angiotensin II and thereby act in opposition to the pro-hypertensive effects of NOX1 and NOX2 (Fig. 2).

The roles of NOX2 and NOX4 in the CNS have also been investigated. Studies using adenoviral-mediated knockdown of NOX2 or NOX4 in the subfornical organ in the CNS reported that both isoforms are required for the full pressor response to angiotensin II in the brain but that only NOX2 was coupled to the water intake response [94].

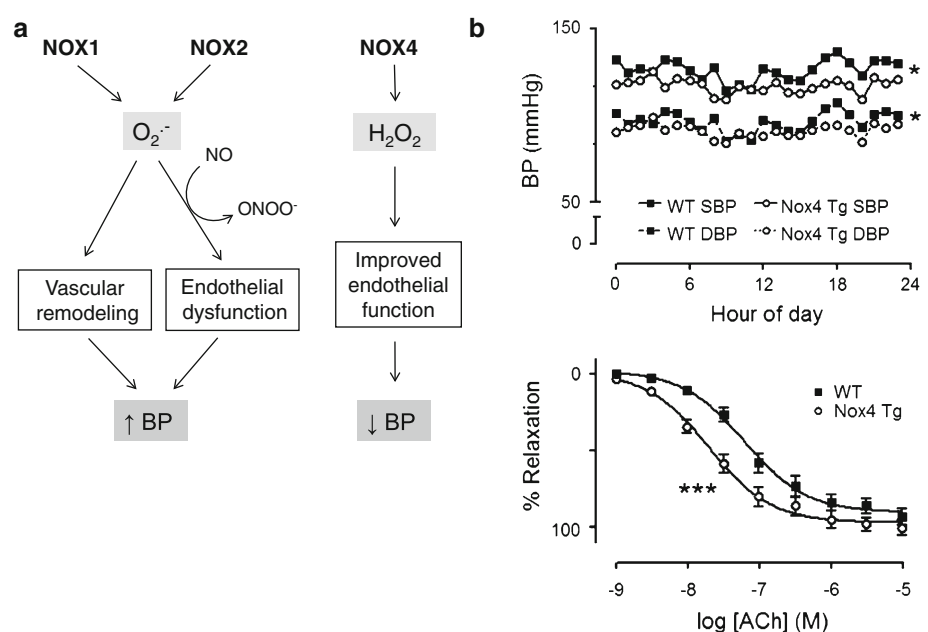
Data on the possible involvement of NOXs in other forms of experimental hypertension are largely restricted to correlative data although it was reported that norepinephrine-induced hypertension was unaffected in NOX1 knockout mice, in contrast to angiotensin II-induced hypertension [37].

In human studies, an association between ‘essential’ (i.e. idiopathic) hypertension and increased vascular NOX expression has been demonstrated in work using resistance arteries from gluteal fat biopsies [112]. Patients with hypertension have also been shown to have endothelial dysfunction in saphenous veins that is attributable to increased NOX-dependent ROS generation [44]. Human genetic studies also revealed polymorphisms of p22^{phox} which appear relevant to hypertension. The C242T, –930(A/G) and –675(A/T) polymorphisms of CYBA, the gene encoding for p22^{phox} , were reported to be associated with increased phagocytic NADPH oxidase activity and increased susceptibility to hypertension [88, 89, 121].

Atherosclerosis

There are several reasons to suspect an involvement of NADPH oxidases in atherosclerosis. Besides being found in all layers of the blood vessel wall [40, 44], the enzyme is present in the macrophage, a central player in atherogenesis [20]. $\text{O}_2^{\cdot-}$ can oxidize LDL and atherogenic oxidized LDL itself stimulates NADPH oxidase [3, 5, 100]. $\text{O}_2^{\cdot-}$ -mediated inactivation of NO promotes endothelial activation and VSMC proliferation, also important steps in atherogenesis [35, 59]. In addition, NOX-modulated redox

Fig. 2 Contrasting vascular effects of NOX1/2 versus NOX4. **a** NOX1 and NOX2 promote endothelial dysfunction and vascular remodelling, at least in part through $\text{O}_2^{\cdot-}$ -dependent inactivation of NO. NOX4 on the other hand enhances endothelial-dependent relaxation through H_2O_2 -dependent hyperpolarization. These effects may contribute to changes in BP. **b** Effects of endothelial-specific NOX4 overexpression on ambulatory BP measured by telemetry (top) and acetylcholine (ACh)-induced relaxation in isolated aortic rings. Tg transgenic mice; SBP systolic BP; DBP diastolic BP. *** $P < 0.001$. Reproduced with permission from [98]



signalling may accentuate both these processes. Interestingly, the propensity of atherosclerotic lesions to form in areas of turbulence such as arterial branch points might also involve mechanical activation of NOXs [38].

Mice with global NOX2 deficiency that were crossed with atherosclerosis-prone apoE^{-/-} mice and fed a high fat diet were found to have no obvious protection against atherosclerosis when examining aortic sinus sections [62]. Similar results were reported in global p47^{phox} knockout mice crossed with ApoE^{-/-} mice [51]. In contrast, Barry-Lane et al. [6] found that total aortic lesion area (from arch to bifurcation) was reduced in p47^{phox} knockout mice crossed with ApoE^{-/-}, suggesting that NOX1 and/or NOX2 may be involved in atherogenesis. Very recently, similar observations have been described in the NOX2/ApoE double knockout mouse with a high-fat diet. [58]. Absence of NOX2 activity did not alter the disease burden within the aortic sinus but was associated with a 50% reduction in lesion area in the region between aortic arch and (iliac) bifurcation. The relevant cellular source was not delineated in these experiments but has been addressed in other work. Khatri et al. [60] employed a ligation-induced model of carotid atheroma in mice with transgenic VSMC-specific overexpression of p22^{phox}. These animals showed enhanced growth of arterial lesions associated with a greater degree of expansive arterial remodelling, possibly a consequence of increased matrix metalloproteinase (MMP) activity. These lesions also showed enhanced angiogenesis (discussed later). Equivalent studies using genetically modified animals for the other vascular isoforms, NOX1 and NOX4, are not presently available.

Very recently, data implicating a role for NADPH oxidase in macrophage trapping in the neointima of lesions have become available. Oxidized LDL-induced changes in macrophage function via CD36 signalling were found to involve ROS-dependent inactivation of the phosphatase SHP2 [93]. This was inhibited by DPI and apocynin, suggesting that it may be NADPH oxidase-dependent although clearly not definitive.

Other indirect evidence comes from the actions of the renin inhibitor aliskiren which was found to reduce aortic sinus plaque area in ApoE^{-/-} mice, seemingly independent of its blood pressure lowering effects, whilst also reducing vascular NOX activity [96]. In contrast, CB-1 cannabinoid receptor inhibition did not reduce atherosclerosis in ApoE^{-/-} mice but did improve aortic endothelial-dependent vasodilation whilst reducing ROS production and NOX activity [109].

In humans, the C242T polymorphism of the CYBA gene (which encodes p22^{phox}) has been linked to increased risk of coronary artery disease or progression of coronary artery disease in certain subgroups with the T allele [14, 15], although other authors have suggested a protective effect of

this allele [29, 55]. These disparate findings may relate, at least in part, to differences in the populations under study and the endpoints used.

Human pathological specimens have also been used for the analysis of NADPH oxidase expression. Azumi et al. [4] found increased immunostaining for p22^{phox} protein in atherosclerotic coronary arteries compared to non-atherosclerotic arteries, and showed that this co-localized with markers for endothelial cells, smooth muscle cells, infiltrating macrophages and adventitial fibroblasts. Later work by Sorescu and colleagues [106] supported these findings and found that p22^{phox} co-localized with NOX2 predominantly in macrophages and particularly in the plaque shoulder regions. mRNA levels for p22^{phox} and NOX2 correlated with lesion severity in this study. In contrast, NOX4 staining was most marked in the vessel media and correlated with VSMC cell density in atherosclerotic lesions. Recently, attention has also focussed upon vascular NOX5 [42, 102]. Guzik et al. studied human coronary arteries from explanted heart tissue and reported that NOX5 mRNA and protein levels were upregulated in atherosclerotic arteries, with staining evident in both the neointima and adjacent medial VSMC. NOX5 contributed significantly to overall NADPH oxidase-generated ROS in these arteries. An unexpected finding from other recent work is that NOX5 may directly activate eNOS, possibly counteracting the effects of NOX's primary product in consuming NO [123].

Ischaemic neovascularization

A significant body of data implicates ROS in the process of angiogenesis and ischaemic neovascularisation, with in vivo evidence suggesting an involvement of NOXs in this process. Using an in vivo sponge implant model in which topical injection of VEGF or sphingosine 1-phosphate stimulated new vessel formation, Ushio-Fukai et al. [114] found that NOX2 knockout mice had reduced VEGF-induced new vessel formation. Later work used a hindlimb ligation model and found that NOX2 knockout mice developed reduced neovascularisation after ischaemia [110]. The ischaemic regions of wild-type mice subjected to hindlimb ischaemia demonstrated an increase in NOX2 protein expression and O₂^{•-} production, with NOX2 localized to infiltrating leucocytes acutely and then in newly formed capillaries at a later stage [110]. Supportive work comes from a retinal neovascularization model in which ROS generation in the ischaemic retina was associated with an increase in NOX2 levels and was reduced by pre-treatment with gp91ds-tat [1].

Recent work investigated the possible contribution of bone marrow-derived cells, using chimeric mice in which

either bone marrow cells and/or resident tissue cells lacked NOX2 [113]. In the setting of hindlimb ischaemia, these studies showed that bone marrow-derived cells were the main source of NOX2 that was involved in post-ischaemic neovascularisation. Ebrahimian and colleagues [27] also studied bone marrow chimeric mice in a similar model but this time with or without diabetes. These authors found that NOX2 was required for post-ischaemic neovascularisation in non-diabetic mice whereas NOX2 impaired neovascularisation in diabetic mice. The authors proposed that the effects of ROS on neovascularisation depended upon the level of ROS that were generated, with high ROS levels in the setting of diabetes being detrimental. The *in vivo* effects of other NOX isoforms remain to be established using specific genetic approaches.

Cardiac hypertrophy and fibrosis

Myocardial hypertrophy occurs in response to chronic stresses such as cardiac overload and involves both mechanical and various neurohumoral stimuli (e.g. activation of the renin-angiotensin system, β -adrenergic activation, cytokines). NOX enzymes are found in multiple cardiac cell types—namely cardiomyocytes, endothelial cells, fibroblasts and vascular smooth muscle cells—as well as in infiltrating inflammatory cells. Furthermore, NOX enzymes, particularly NOX1 and 2, are activated by known pro-hypertrophic stimuli such as angiotensin II, tumour necrosis factor α (TNF α) and mechanical stretch [32, 53, 75, 76, 120]. It is, therefore, perhaps not unexpected that they are involved in mediating cardiac hypertrophy.

Using a model of experimental cardiac hypertrophy induced by chronic subpressor infusion of angiotensin II, Bendall et al. [9] made the initial observation that NOX2 was critical for this response since hypertrophy was markedly inhibited in NOX2 knockout mice. This was accompanied by an inhibition of interstitial cardiac fibrosis in NOX2 knockout mice. Later studies provided evidence that this effect on hypertrophy involved cardiomyocyte NOX2. Satoh and colleagues [101] studied mice with a cardiomyocyte-specific deletion of Rac1 and found that these animals had markedly blunted hypertrophic responses to angiotensin II infusion. As discussed above, Rac1 is required for NOX2 activation and these authors showed the Rac1 deficient mice also had deficient activation of NOX2 together with reduced activation of ASK1 and NF- κ B.

Early studies in experimental models of aortic constriction-induced pressure overload hypertrophy found a progressive increase in left ventricular NOX-dependent ROS generation which was associated with an increase in

expression of NOX2 and related oxidase subunits and was accompanied by MAP kinase (MAPK) activation—suggesting that these may be downstream targets of NOX-generated ROS [74]. However, studies in NOX2 knockout mice found that this subunit was not essential for the development of hypertrophy. These animals developed a similar degree of hypertrophy and similar increases in molecular markers of hypertrophy as wild-type littermates [13]. Similar results were obtained by an independent group [86], indicating that there are significant differences between angiotensin II and pressure overload-induced hypertrophy with respect to an essential requirement for NOX2.

Despite the lack of requirement for NOX2 for the development of pressure overload hypertrophy, it was found that NOX2 knockout mice developed less interstitial fibrosis and contractile dysfunction than wild-type littermates [41]—indicating a dissociation between hypertrophy *per se* and fibrosis or contractile dysfunction. In line with this, NOX2 has been shown to be critical for the development of interstitial cardiac fibrosis in models of high-dose angiotensin II infusion [57] or activation [111] as well as in aldosterone/salt hypertension [57], even though it had no effect on the extent of hypertrophy. These results may indicate cell type-specific effects of NOX2 in the development of cardiac hypertrophy and fibrosis.

In early studies of the role of NOX2 in pressure overload hypertrophy, Byrne et al. [13] found that NADPH oxidase activity increased to similar levels in NOX2 knockout and wild-type mice, and that this was the result of an increase in NOX4 levels. It was accordingly speculated that NOX4 might compensate for NOX2 in this setting. However, as mentioned earlier, the two NOX isoforms differ significantly in terms of their regulation. In addition, whereas activated NOX2 is located at the sarcolemma [49], NOX4 is found in an intracellular perinuclear location [122]. Recent studies from our laboratory have investigated the role of NOX4 using newly generated mouse models with a deletion of NOX4 or a cardiomyocyte-targeted increase in NOX4 expression. Neither model developed significant basal changes in cardiac hypertrophy or function [122]. After imposition of chronic pressure overload, however, NOX4 KO mice developed significantly greater hypertrophy and contractile dysfunction than controls whereas NOX4 transgenic mice exhibited protection against hypertrophy and failure. The underlying mechanism was found to be a NOX4-dependent preservation of myocardial capillary density during pressure overload, which involved an augmentation of stress-induced cardiomyocyte hypoxia-inducible factor 1 (HIF1) activation and the release of VEGF, resulting in increased paracrine angiogenic activity. Whereas an insufficient increase in capillary number

relative to increase in cardiomyocyte size during hypertrophy is thought to promote pathological remodelling of the heart [104], the NOX4-dependent preservation of capillary density may act to counteract this (Fig. 3). These results on NOX4 along with the previous studies on NOX2 indicate that the two isoforms exert markedly different effects during pressure overload-induced cardiac hypertrophy, with NOX2 being detrimental and NOX4 beneficial.

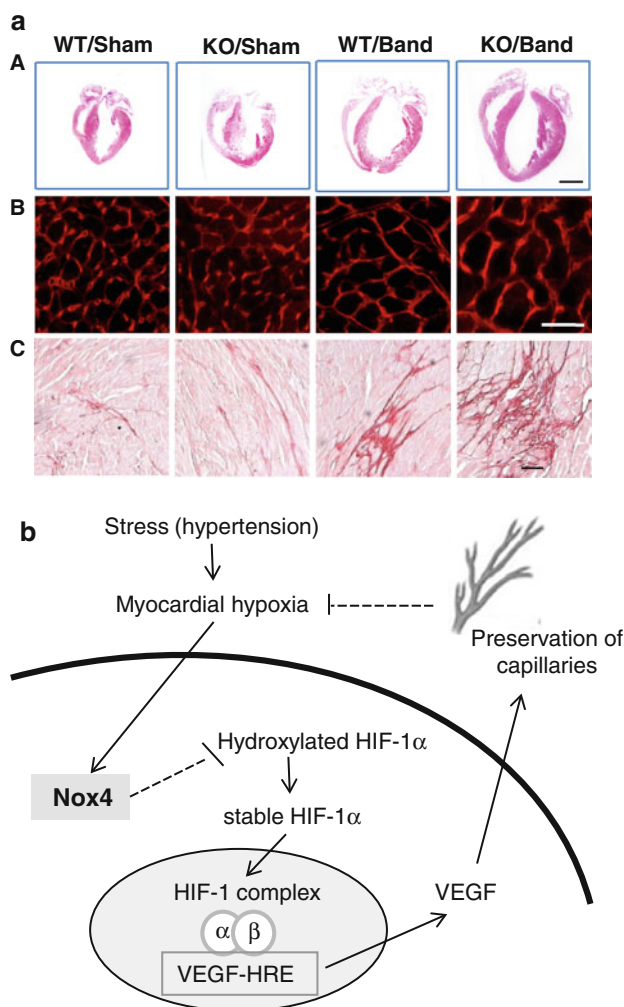


Fig. 3 Protective role of NOX4 during pressure overload cardiac hypertrophy. **a** Representative images from NOX4 knockout mice or wild-type littermates (WT) subjected to pressure overload by aortic constriction. *Panel A* shows whole heart sections (scale bar 2 mm); *panel B* shows myocardial sections stained with wheat-germ agglutinin to show cardiomyocyte area; *panel C* shows myocardial sections stained with Picrosirius Red to show fibrosis (scale bars 20 μ m). NOX4 KO mice developed greater hypertrophy, fibrosis and remodelling than WT after pressure overload. Reproduced with permission from Zhang et al. [122]. **b** Schematic illustrating the mechanism underlying protective effects of NOX4. Upregulation of NOX4 enhances HIF1 activation and the release of VEGF which exerts paracrine effects to promote preservation of myocardial capillary density

Myocardial infarction, post-MI remodelling and heart failure

Post-MI cardiac remodelling refers to structural and functional alterations that occur in response to MI and predispose to the development of heart failure [95]. Features of post-MI cardiac remodelling include infarct expansion, hypertrophy of non-infarcted myocardium, ventricular dilatation, a change in chamber shape to a more spherical geometry, and systolic and diastolic dysfunction. This is associated with an increase in interstitial and perivascular fibrosis, other alterations in the extracellular matrix, and cardiomyocyte hypertrophy and apoptosis [107].

Although some of the determinants of post-MI myocardial remodelling, such as infarct size and neurohormonal status [18, 24, 45, 46], are the targets of currently available therapies, there is still a need for new therapeutic targets. Oxidative stress is implicated in the development of post-MI remodelling and could, therefore, be a therapeutic target [48, 64]. Indeed, various antioxidant agents have been shown to ameliorate remodelling after MI in rodents [61, 90], while genetically modified mice with increased activity of endogenous antioxidant systems were also protected against post-MI ventricular dilatation and dysfunction [105].

Specific involvement of NOX enzymes is suggested by clinical data demonstrating an upregulation of NOX2 in cardiomyocytes in patients who had died after MI [65]. An increase in the expression of p22^{phox} and NOX2 proteins has also been found in the infarcted region of rats after experimental myocardial infarction [34]. More direct evidence was derived from studies in genetically altered mice. Early work showed that acute infarct size after in vivo myocardial ischaemia/reperfusion was not significantly different between NOX2 knockout mice and wild type [50]. However, studies by Bell and colleagues [8] reported that NOX2 mediates ischaemic cardiac preconditioning in isolated hearts, suggesting that it could affect infarct size under some circumstances. Later studies have investigated the role of NOX2 in post-MI remodelling. Looi and colleagues [81] studied NOX2 knockout mice subjected to permanent left coronary ligation and found that post-MI remodelling at 4 weeks was significantly reduced despite no difference in infarct size (Fig. 4). The improvement in left ventricular volumes and function was accompanied by a reduction in cardiomyocyte hypertrophy, apoptosis and interstitial fibrosis in the non-infarcted myocardium along with a decrease in mRNA levels of connective tissue growth factor (CTGF), procollagen I and ANF and lower MMP activity. Strong support for such a role of NOX2 comes from independent studies that examined post-MI remodelling in p47^{phox} knockout mice [23]. These animals (which lack NOX2 activity) were also protected against

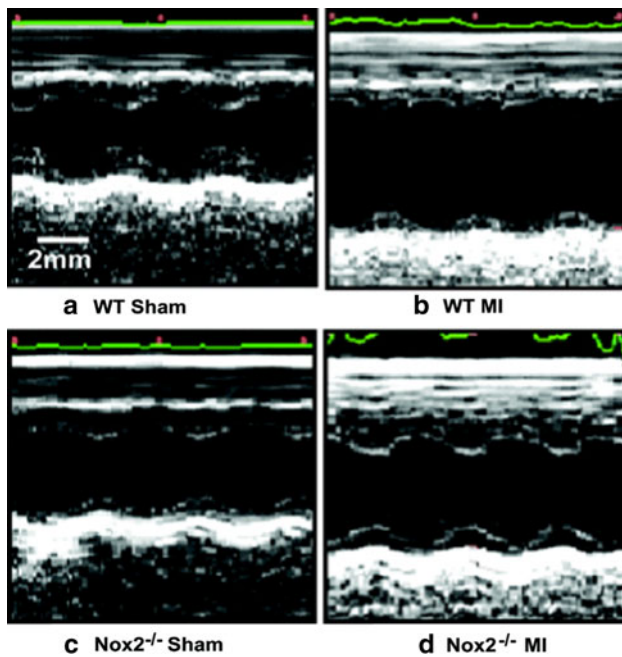


Fig. 4 Effect of NOX2 deletion on post-MI remodelling. Representative M-mode echocardiographic images from NOX2 knockout mice or wild-type littermates (WT) subjected to left coronary ligation or sham ligation surgery. The extent of LV dilatation and reduction in fractional shortening 4 weeks after MI is significantly reduced in the NOX2 knockout group compared to WT. Reproduced with permission from Looi et al. [81]

post-MI remodelling and dysfunction with a similar pattern of underlying changes as found by Looi and colleagues. In contrast to these studies, Frantz et al. [31] failed to find a similar beneficial effect of NOX2 and instead reported a higher mortality in NOX2 knockout mice. Some caveats to this last study's design are pertinent, e.g. the lack of littermate controls which can be a confounding factor [39], and the absence of a sham-operated group. Overall, NOX2 appears to play an important role in post-MI adverse cardiac remodelling.

Very recently, NOXs within the central nervous system have been shown to have important effects on post-MI cardiac remodelling. It has been known for some time that superoxide production within certain nuclei of the brain (including the paraventricular nucleus) is implicated in mediating the sympathetic activation that occurs after MI and which predisposes to heart failure [73, 79]. Injection into the cerebral ventricles of superoxide dismutase (in a viral carrier) led to reduced activation of neurons in these CNS nuclei after MI [79], and resulted in reduced apoptosis and improved cardiac function and survival post-MI [80]. Recent studies showed that NOX4 was the most highly expressed NOX isoform within the paraventricular nucleus (PVN) after experimental murine MI and that siRNA-mediated knockdown of this isoform in the PVN reduced

sympathetic outflow from the brain, reduced peri-infarct apoptosis and improved cardiac performance [54].

The main adverse clinical consequence of cardiac remodelling is the development of overt heart failure. Clinical data are available regarding NADPH oxidases in end-stage heart failure, mostly derived from explanted failing hearts at the time of transplantation. Our group demonstrated that NADPH oxidase activity was significantly upregulated in such hearts compared to non-failing hearts and that this was associated with a greater membrane translocation of p47^{phox} [49]. Similar findings were made by Maack and colleagues [82], who also described increased Rac1 translocation from cytosol to membrane, associated with elevated rac1-GTPase activity. Downstream pathways have also been examined in this context and there is evidence of increased activation of ERK1/2 and p38MAPK in parallel with increased NOX activity in end-stage failing hearts, both in the left and right ventricles [91]. It has been suggested that the right ventricle may be more susceptible to increased oxidative stress due to poorer antioxidant defence activation and that this may be relevant in the development of overt heart failure [11]. An increase in NOX2-dependent ROS generation associated with endothelial dysfunction has also been reported in saphenous veins obtained from patients with heart failure [26], suggesting that increased NOX2 activation may have wider systemic effects in the setting of heart failure. In an experimental model of diastolic heart failure, use of a pharmacological agent to enhance eNOS activity produced attenuation of diastolic dysfunction and reduced levels of NOX2 subunits, suggesting a role for oxidative stress in modulating cardiac function in this setting too [118].

Conclusions

NADPH oxidases are major sources of ROS in the heart and vasculature that appear to have an important role in the pathogenesis of several cardiovascular conditions. While clinical evidence for their importance is largely correlative, more robust data derive from experiments in gene-modified animals. These studies have largely focused on NOX2 and NOX1, whereas the roles of NOX4 have been less clear. Work to date indicates that NOX1 and/or 2-mediated signalling may be detrimental in hypertension, atherosclerosis, cardiac hypertrophy and remodelling. However, recent work suggests that NOX4 may play a protective role in the heart subjected to chronic pressure overload as well as in the vasculature in the setting of hypertension. Additional research on the isoform-specific roles of the NOXs and on the specific downstream targets that they affect may define suitable therapeutic targets for cardiovascular diseases.

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